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Examining Intrinsic Thalamic Resting State Networks Using Graph Theory Analysis : Implications for mTBI detection.

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Abstract— **A major challenge associated with understanding mild traumatic brain injury (mTBI) is the absence of biomarkers in standard clinical imaging modalities. Furthermore, the inhomogeneity of mTBI location and intensity, combined with latent symptoms further complicates identification and treatment. A growing body of evidence suggests that the thalamus may be injured or susceptible to change as the result of mTBI. A significant number of connections to and from cortical, subcortical, cerebellar and brain stem regions converge at the thalamus. Furthermore, the thalamus is also involved with information processing, integration and the regulation of specific behaviors. We use graph theory analysis to evaluate intrinsic functional networks of the left and right thalamus in mTBI subjects (N=15) and neurologically intact healthy controls (N=12). We also explore neural correlates of the thalamic network architecture with clinical assessments. Our results suggest the presence of distinct unilateral thalamic differences in mTBI subjects. We also observe correlations of the thalamic changes with clinical assessments. The findings from this study have implications for functional networks in the thalamus and its projections for application as a potential biomarker for mTBI detection.**

I. Introduction

Traumatic brain injury (TBI) is an important public health care concern with an estimated 1.7 million cases reported annually and a corresponding financial burden of approximately $56 billion for lifetime total cost of treatment in the civilian population [1,2]. In the military population, it is estimated that there are up to 360,000 service members who suffer from TBI [1,3]. Mild TBI (mTBI) is the most prevalent form of TBI and accounts for 89% of all reported TBI incidents [3]. Assessing the effects of mTBI within individuals is challenging and sometimes controversial because unlike stroke or severe TBI where clear locations of neurological infarct are visible, the focal abnormalities are often not detected using standard clinical imaging modalities (CT and MRI) in cases of mTBI [4,5]. Due to the inhomogeneity of mTBI damage location and intensity, individuals frequently experience varied symptoms, some of which are transient [6,7]. Others symptoms may occur at a higher latency from the onset of injury [6,7]. These symptoms contribute toward disruptions to activities of daily living and increase the risk of chronic impairment.

Diffuse axonal injury in white matter (WM) tracts has been identified as a common underlying cause for deficits associated with TBI [8,9,10]. However, there is a growing body of evidence suggesting that TBI could induce thalamic injury, classically identified as gray matter (GM) tissue, leading to abnormal activations and subsequent impairment [10,11,12]. The thalamus has been labeled as the 'relay station' of the brain and is responsible for transmitting information to and from multiple cortical, subcortical, cerebellar and brain stem regions [13,14]. Several major pathways that involve cognitive, motor, sensory and memory related functions converge through the thalamus. Although the thalamus is classified as GM, in fact it contains many white-matter axons connecting to and passing through it. It is involved with processing and integrating information, and in addition also regulates specific behaviors such as alertness, mental state, and some motor and sensory functions [13,14].

The main objective of this study is to examine and quantify the intrinsic functional networks of the thalamus at resting state, using measurements of graph theory. We also explore the association between thalamic network architecture and clinical assessments. We hypothesize that changes within the intrinsic networks of the thalamus associated with mTBI can be detected and quantified at resting state.

II. Methods

A. Subjects

A total of 15 USA military active duty male subjects (age = 25.6 ± 4.4 years) who had returned from Afghanistan or Iraq and were clinically diagnosed with mTBI (143.3 ± 85.4 days since injury) were recruited for this study. The control subjects consisted of 12 individuals (M = 9, F = 3, age = 26.4 ± 5.8 years) with no history of brain trauma. All subjects gave written informed consent to participate in the study, which was approved by the institutional review board of the Walter Reed National Military Medical Center

B. Clinical Assessments

The mTBI subjects were administered 12 neuropsychological tests to assess neuropsychologic symptoms and neurocognitive function. These tests were the Figural Fluency Test, Delis-Kaplan Executive Function System (DKEFS), Conners' Continuous Performance Test

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14. ABSTRACT

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(CPT), California Verbal Learning Test (CVLT), Weschsler Adult Intelligence Scale (WAIS), Weschsler Test of Adult Reading (WTAR), Personality Assessment Inventory (PAI), 36-Item Short Form Health Survey (SF36), Brief Symptom Inventory (NBSI), Post Traumatic Stress Disorder (PCL-C) and the Automated Neuropsychological Assessment Metrics (ANAM).

C. Data Acquisition and Experiment Paradigm

Images were acquired on a Discovery 750 MRI scanner [GE Healthcare, Waukesha, WI] with a 32-channel phased array head coil [MR Instruments, Inc. Minnesota, MN]. Whole brain functional MRI (fMRI) data were acquired with an EPI pulse sequence in the sagittal plane (TE/TR = 25/2000 ms, FA = 60°, in plane resolution = 3.75×3.75×4mm^3, matrix size 64×64 and FOV 240mm). A T1-weighted anatomical scan of the entire brain (3D BRAVO sequence: TR/TE = 6.7/2.5 ms, FA = 12°, Resolution = 0.5 × 0.5 × 0.5 mm^3) was also acquired. In this paper, we present results from a resting state fMRI task, which was a subset of a larger number of anatomical and functional scans. During the resting state task, subjects were asked to lie supine in the scanner with their eyes closed for 6 minutes.

D. Data Analysis

The AFNI software package [15] was used for 3D head movement correction and to perform temporal interpolation to correct for slice time acquisition differences. The first 3 time points were discarded to account for magnetic field equilibrium. Data sets were transformed into NIfTI format [http://nifti.nimh.nih.gov]. Individual subject segmentation of cortical and subcortical brain regions was performed using Free Surfer based on the MNI305 atlas [16,17]. Using the resulting individual segmentation information, raw time series information was extracted from all voxels of the thalamus for each subject.

Subsequent analysis was performed using custom written MATLAB scripts [Mathworks Inc., Natick MA]. Data were temporally filtered using a finite impulse response, zero phase distortion bandpass filter (0.01 – 0.1Hz)[18,19]. The linear correlation coefficient was calculated between voxels using the methods described in Eguiluz, *et. al.* [20]. A correlation threshold of 0.7 was applied to the correlation matrix which was subsequently binarized to create an unweighted and undirected network [21]. Next, graph theoretical analysis involving the measurement of the mean cluster coefficient, efficiency, density, characteristic path length, and degrees was performed using the brain connectivity toolbox [21]. The mean cluster coefficient denotes the fraction of a specific node's neighbors that are also neighbors with one another and is indicative of the robustness of a specific network [20,21]. Efficiency is a measure of the traffic capacity of a network and dictates how reliably information is able to flow within a particular network [20,21]. Density represents the total number of connections within a specific network [20,21]. The characteristic path length is the average shortest connection between all pairs of nodes in the network [20,21]. The Degrees characteristic is the number of links connected to a specific node [20,21].

An independent samples t-test was performed at a confidence interval of 95% using SPSS [IBM Corp., Somers, NY, USA] for each of the graph theory measurements to compare the mTBI subjects and controls. Pearson's correlation was calculated using SPSS to assess the relationship between clinical assessments and thalamic network architecture. For clinical assessments that had correlations that were less than 0.5 with the respective thalamic network measurements were discarded.

III. Results

A. Comparison of Group Results

Figure 1 presents an example of intrinsic connectivity networks in the right thalamus for an individual subject from each group. The red dots indicate voxels in the thalamus and the black lines represent connections between voxels.

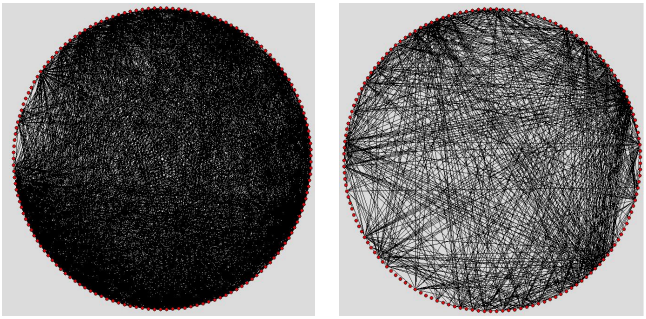

Figure1 : Example comparing intrinsic connectivity network in right thalamus for single control (left) and single mTBI subject (right).

The results from the *t-test* analysis suggests significant differences between groups for the mean cluster coefficient ($F = 6.596$, $df = 25$), $p < 0.017$, density ($F = 4.956$, $df = 25$), $p < 0.035$, degrees ($F = 5.638$, $df = 25$), $p < 0.026$ and characteristic path length ($F = 4.82$, $df = 25$), $p < 0.038$ measurements of the right thalamus. The left thalamus did not reveal any statistically significant results at $p < 0.05$.

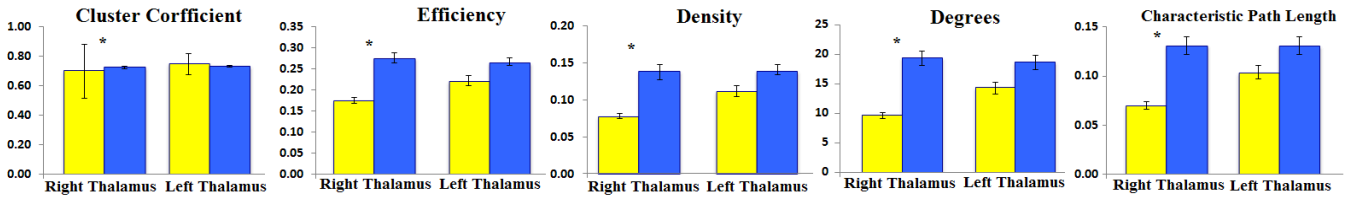

Figure 2 : Average (mean) for both the right and left thalamus for each of the 5 graph theory metrics measured with ±1 SEM comparing mTBI (yellow) and controls (blue). The asterix (*) denotes statistical significance at $p < 0.05$

B. Assessment of Clinical Correlations with Graph Theory Functional Network Measurements

TABLE I. CORRELATION OF CLINICAL ASSESSMENTS WITH GRAPH THEOERY ANALYSIS METRICS FOR RIGHT THALAMUS

	Mean Cluster Coefficient		Characteristic path length		Degrees		Density		Efficiency	
Right Thalamus	R^2	*p* val	R^2	*p* val	R^2	*p* val	R^2	*p* val	R^2	*p* val
CPT Reaction Time					0.527	0.044	0.515	0.049		
CPT Number Omissions	0.681	0.005								
NBSI Change in taste and/or smell					0.591	0.02	0.589	0.021		
NBSI Loss of appetite or increase appetite					0.558	0.031				
NBSI Headaches							0.531	0.042		
NBSI Difficulty making decisions							0.051	0.052		
NBSI poor concentration, cannot pay attention, easily distracted									0.537	0.039
PCL-C difficulty concentrating					0.508	0.053	0.504	0.055	0.564	0.029
PAI mania							0.822	<0.001	0.614	0.015
SF36 Q36 My health is excellent			-0.55	0.034						
SF36 Q15 Physical health limits work & activity					0.534	0.04	0.506	.054		
SF36 Q2 Self rating of present health					0.58	0.023	0.587	0.021		
SF36 Q19 Emotional problems affecting work or other activities									0.535	0.04
CVLT II Total accurate Forced Choice	-0.582	0.023	0.606	0.017						
CVLT II Delayed Recall Total for Recognition Discrimination									-0.526	0.044

TABLE II. CORRELATION OF CLINICAL ASSESSMENTS WITH GRAPH THEOERY ANALYSIS METRICS FOR LEFT THALAMUS

	Mean Cluster Coefficient		Characteristic Path Length		Degrees		Density		Efficiency	
Left Thalamus	R^2	*p* val	R^2	*p* val	R^2	*p* val	R^2	*p* val	R^2	*p* val
DKEFS Total Score	0.0532	0.041								
SF36 Q36 My health is excellent	0.517	0.048	-0.53	0.042						
SF32 Q2 Self rating of present health					-0.507	0.054			-0.516	0.049
SF36 Q Q12 Health limits bathing or dressing					-0.536	0.039	-0.517	0.048		
CVLT II Forced Choice Total Accurate	-0.812	< 0.001	0.812	< 0.001						

IV. DISCUSSION

The significant differences between the mTBI and control subjects for the right thalamus suggest that the use of graph theory analysis methods are able to adequately quantify intrinsic network architecture within the thalamus. Furthermore, the results indicate that the analysis is also sensitive to differences in network architecture. Overall, the results support our initial hypothesis.

The research and applications of functional connectivity analysis have largely been focused on representing inter-region relationships. Such analyses provide information on large-scale networks which are beneficial for severe and moderate TBI in which clear locations of injury are present. However, large-scale inter-region analysis may not be sensitive enough to detect subtle local regional changes which are present in mTBI. Therefore, our analysis of intrinsic functional networks within a specific brain region is unique as compared to common inter region analysis. Understanding local network organization and the effects of mTBI on network architecture presents itself as a potential biomarker for individual subject assessment. Graph theory is a model free analysis method which has very minimal *a prior* assumptions and provides a relatively unbiased approach of assessing functional connectivity [20,21]. Such a feature is of particular benefit in assessing mTBI due to the variations of injury location and intensity across individuals.

A study performed by Tang *et al.* [10] that examined functional connectivity of the resting state brain using seed regions of the thalamus is in agreement with our findings. The unilateral anomalies detected in the thalamic networks suggest the presence of increased variability in the resting state network, and even a reduction in the resting state network as seen by the overall lower measurements for the mTBI subjects. Resting state networks are known to naturally deactivate or disappear altogether during the performance of voluntary or intense tasks [22]. However, reduction in the resting state network at rest has been associated with interruptions in cognitive states. Several studies have consistently found strong correlations between disruptions in the resting state networks and neurocognitive pathologies such as schizophrenia, Alzheimer's disease and attention deficit hyperactive disorder [22-26]. Many of these

neurocognitive pathologies affect various associative and executive regions that are known to have projections to the thalamus. This feature shows some overlap with the findings from our mTBI clinical assessments, especially in the correlated assessments specific to features of perception, mental state and self awareness. The correlations of density and degrees in the right thalamus that correspond with disruptions in smell, taste and appetite suggest possible changes in the thalamic nuclei as the result of possible injury. Research which examined thalamic lesions in stroke patients have reported compromised smell, taste and loss of appetite [27]. The correlations of density and degree with SF35, Q15 also suggest disruptions in physical ability, however it is not clear from the present data if these are strictly due to disruptions in motor control and coordination or if these disruptions are the result of cognitive and emotion impairments.

An interesting observation was seen in the efficiency measurement of the thalamic networks. Efficiency was not statistically significant for the left and right thalamic networks between mTBI and controls. This suggests that information was still able to flow within the thalamus. However, differences in network architecture as reflected by the mean cluster coefficient, reduced density and degrees, present the possibility that local reorganization within the thalamus has occurred as the result of mTBI. Although our results are promising, more research is needed. In particular, future work should employ a larger sample size to further validate the findings from this study. In addition, the combination of Diffusion Tensor Imaging (DTI) measures such as fractional anisotropy and fiber tractography would strengthen the understanding of the intrinsic functional networks and their respective architecture, and could provide further insight into thalamic projections.

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